Esophageal Morphology from Linxian, China

Squamous Histologic Findings in 754 Patients

Sanford M. Dawsey, M.D., Klaus J. Lewin, M.D., F.R.C.Path.,† Fu-Sheng Liu, M.D.,‡ Guo-Qing Wang, M.D.,§ and Qiong Shen, M.D.‖*

Background. **Linxian, China, has one of the highest rates of esophageal cancer in the world. Other authors have described high prevalences of histologic esophagitis, atrophy, and dysplasia in Linxian and have suggested that these findings may represent precancerous lesions in this population. In 1987, a new endoscopic survey allowed the authors to make an independent study of esophageal histology in Linxian.**

Methods. **There were 1567 satisfactory squamous esophageal biopsies available from 754 patients. These biopsies were classified as normal, atrophy, acanthosis, esophagitis, squamous dysplasia, or squamous cancer.**

Results. **Classified by their worst diagnosis, 56.5% of the 754 patients had normal mucosa, 0.0% atrophy, 11.5% acanthosis, 4.6% esophagitis, 22.7% squamous dysplasia, and 4.6% squamous cancer.**

Conclusions. **The results show a different distribution of esophageal squamous diagnoses than has been reported previously from this population. The authors believe that the major reason for this discrepancy was differences in histologic criteria. In this survey, seemingly small differences in criteria could cause large differences in apparent disease prevalence; this was especially true for esophagitis. By the criteria used in this study, histologic esophagitis and atrophy are uncommon findings in Linxian, raising questions about their significance as precursor lesions of esophageal cancer in this population.** ***Cancer*** **1994; 73:2027–37.**

Key words: endoscopy, pathology, esophagus, normal histology, esophagitis, squamous dysplasia, precancerous conditions.

China has the highest esophageal cancer mortality rates in the world, and Linxian, Henan Province, has one of the highest rates of esophageal cancer in China.[1] Since 1983, the Cancer Institute of the Chinese Academy of Medical Sciences and the U.S. National Cancer Institute have collaborated to carry out two prospective Nutrition Intervention Trials in Linxian to test the hypothesis that nutritional supplementation can affect the incidence and mortality of esophageal cancer.[2] As a part of this collaboration, an endoscopic survey was performed in 1987 on patients in the Dysplasia Trial, a study limited to individuals with a previous cytologic diagnosis of esophageal dysplasia. This endoscopic survey allowed us to examine a wide range of squamous esophageal biopsies from this high-risk population. This paper describes and illustrates the histologic findings of this survey and compares our results with those of previous authors.

Materials and Methods

Endoscopic Survey

Details of the Dysplasia Trial have been described previously.[2] Briefly, 12,877 Linxian residents were screened by esophageal balloon cytology in 1983.[3] Of those screened, 3318 with a cytologic diagnosis of dysplasia were randomized into the treatment or the placebo arm of a six-year double-blind prospective nutrition intervention trial. Active intervention, consisting of daily tablets containing 26 vitamins and minerals at two to

From the *Cancer Prevention Studies Branch, National Cancer Institute, Bethesda, Maryland 20895; the †Department of Pathology, UCLA Center for the Health Sciences, Los Angeles, California; the ‡Departments of Pathology and §Chest Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China; and the ‖Department of Precancerous Studies, Henan Medical University, Zhengzhou, Henan Province, China.

Supported by contracts NO1-CP-95616 and NO1-CP-05634 of the National Cancer Institute.

The authors thank Andrew Nuland and Linda Cranston of Westat, Inc., Rockville, MD, for computer programming support.

Address for reprints: Sanford M. Dawsey, M.D., Cancer Prevention Studies Branch, National Cancer Institute, Executive Plaza North, Room 211, 9000 Rockville Pike, Bethesda, Maryland 20892.

Accepted for publication December 13, 1993.

three times U.S. Recommended Daily Allowances, began on May 1, 1985.

In November and December, 1987, after 30 months of active intervention, 833 dysplasia trial patients underwent endoscopy. The study was approved by the Human Research Review Committee of the Cancer Institute of the Chinese Academy of Medical Sciences, and informed consent was obtained from each patient before the procedure.

During endoscopy, the entire esophagus and stomach were visually examined. If a focal lesion was found, one or more 2.8-mm clinical biopsies were taken for clinical diagnosis. If, after these biopsies were removed, enough of the focal lesion was still left, two 2.8-mm research biopsies also were taken from this site. If the focal lesion was removed completely by clinical biopsies or if no such lesion was present, the research biopsies were taken from the middle third of the esophagus.

The clinical biopsies were fixed immediately in 10% buffered formalin. The research biopsies were oriented on filter paper, incubated for 1 hour for tritiated thymidine incorporation, and then fixed. One of each pair of research biopsies was fixed in 10% buffered formalin, and the other was fixed in 95% ethanol. All of the biopsies were embedded in paraffin, cut in 5-μm sections, and stained with hematoxylin and eosin.

The biopsy slides were read jointly by three pathologists (S.M.D., K.J.L., F.S.L.) without knowledge of the patient's history, treatment group, or the visual endoscopic findings.

Histologic Categories

The histologic criteria were based on previous descriptions.[4–7] After reviewing several hundred biopsies, we modified some criteria, leading to the following definitions:

Normal. There was a well-oriented stratified squamous epithelium, containing a basal zone and a superficial zone, with or without underlying lamina propria (Fig. 1). The basal zone was defined as including the cells from the basal lamina to the level where the nuclei were separated by one nuclear diameter.[8] The superficial zone, including all of the epithelium above the basal zone, was composed of spinous cells that became flattened progressively toward the surface. Most biopsies showed some mature squamous cells with abundant clear cytoplasm, an appearance we called clear cell change. Nearly all biopsies showed a few lymphocytes scattered in the epithelium, without evidence of associated tissue damage. The epithelial thickness was between that of atrophy and acanthosis, as defined below. There was no surface keratinization, parakeratosis, or evidence of esophagitis, squamous dysplasia, or squamous cancer, as defined below.

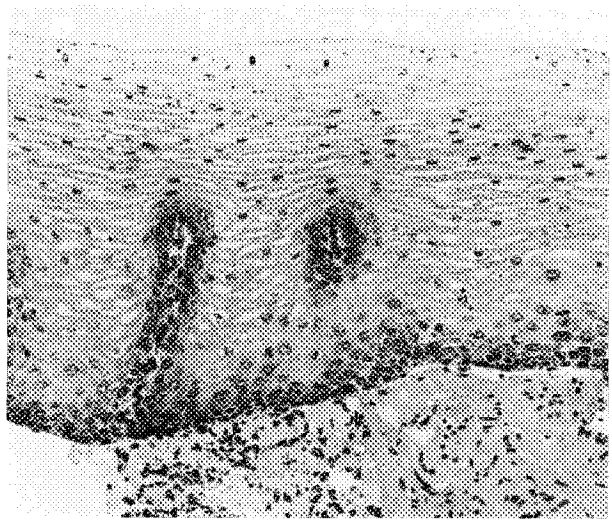

Figure 1. Normal squamous mucosa. The papillae are confined to the lower two thirds of the epithelium, and the basal zone is only a few cells thick (original magnification, ×200).

Atrophy. A well-oriented portion of the epithelium was less than or equal to 10-cells thick, and there was no convincing evidence of loss of surface epithelium during the biopsy procedure or of granulation tissue in the underlying lamina propria. In addition, there was no evidence of esophagitis, squamous dysplasia, or squamous cancer.

Acanthosis. A well-oriented portion of the epithelium, measured from basal lamina to surface, was greater than or equal to 0.5-mm thick (Fig. 2). Using our Olympus BHS microscope (Olympus Corporation, Lake Success, NY) with 10× widefield eyepieces and a 20× objective lens (×200, total magnification), acanthotic epithelia filled half or more of the microscopic field. There was no evidence of esophagitis, squamous dysplasia, or squamous cancer.

Esophagitis. One or more of the following three criteria were present: (1) the lamina propria papillae extended into the upper third of the epithelium and the basal zone thickness was more than 15% of the epithelial thickness (Figure 3); (2) there was focal or diffuse infiltration of the epithelium by polymorphonuclear leukocytes (≥ 2 cells/tissue section) or eosinophils (≥ 1 cell/tissue section) (Figure 4); and/or (3) there was a dense non-follicular infiltrate of mononuclear inflammatory cells and/or an easily recognized infiltrate of neutrophils in the lamina propria (Figure 5). Both elongated papillae and basal cell hyperplasia were required for criterion 1; this criterion was not used in biopsies from the distal 2.5 cm of the esophagus, because these features can be present in normal biopsies from this

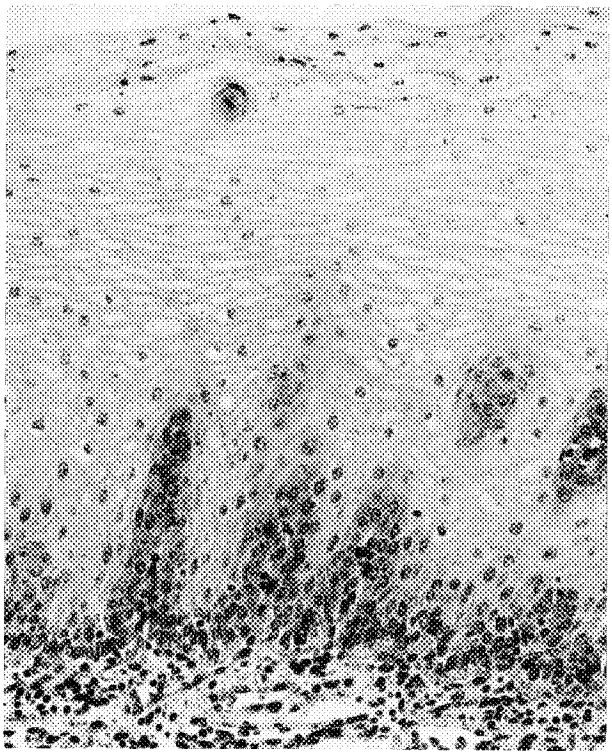

Figure 2. Acanthosis. The epithelium is thicker than normal mucosa (original magnification, ×200).

area (5). Only criteria 2 and 3 were used in biopsies showing squamous dysplasia or squamous cancer, because basal zone thickness could not be evaluated in these settings. Esophagitis was graded as mild, moderate, or severe based on the amount of inflammation

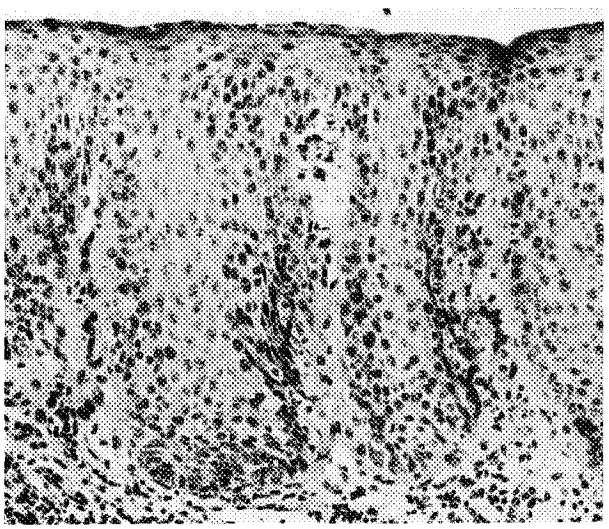

Figure 3. Esophagitis. This biopsy resembles the reflux esophagitis commonly seen in western countries, with characteristic marked elongation of lamina propria papillae (original magnification, ×200).

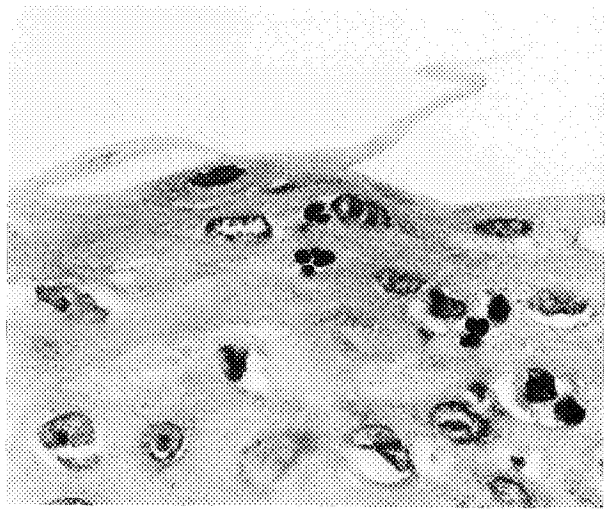

Figure 4. Esophagitis. The epithelium is infiltrated by clearly recognizable polymorphonuclear leukocytes (original magnification, ×1000).

present. If only criterion 1 was present, the esophagitis was graded as mild. All biopsies with histologic erosion or ulceration of the epithelium were graded as severe.

Squamous dysplasia. Nuclear atypia (enlargement, pleomorphism and hyperchromasia), loss of nor-

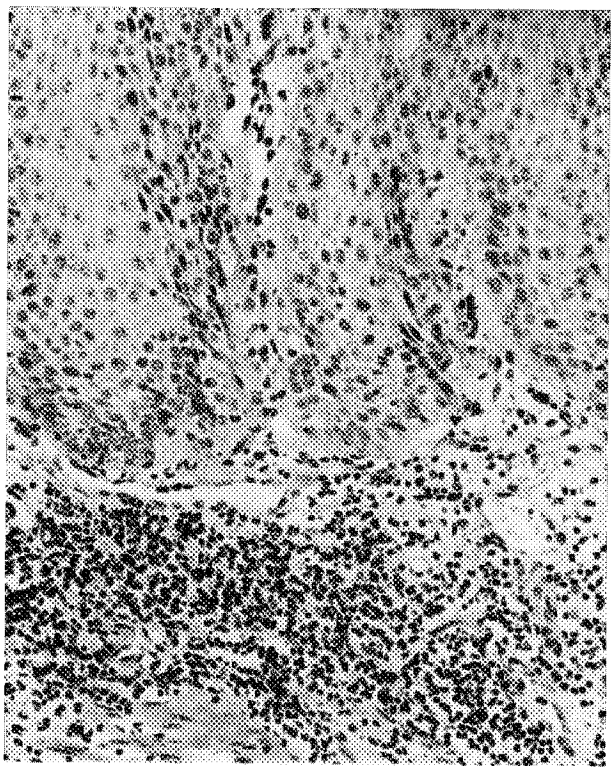

Figure 5. Esophagitis. There is a dense nonfollicular infiltrate of mononuclear inflammatory cells that is confined to the lamina propria (original magnification, ×200).

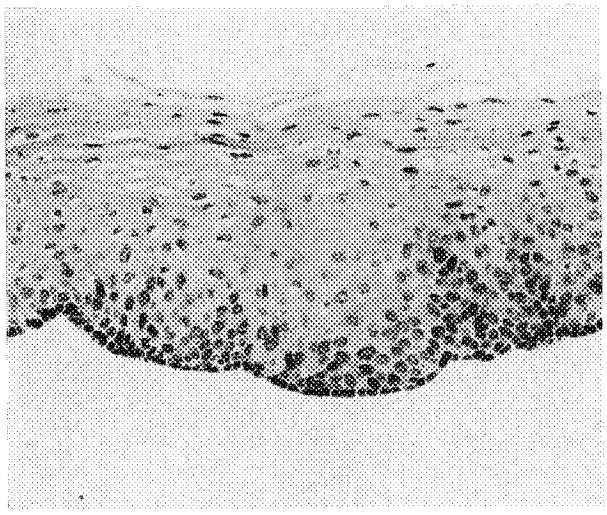

Figure 6. Mild squamous dysplasia. There are enlarged, hyperchromatic nuclei in the lower third of the epithelium that have lost their basal orientation. Higher in the epithelium, normal squamous maturation is evident (original magnification, ×200).

mal cell polarity, and abnormal tissue maturation were present, without invasion of epithelial cells through the basement membrane. In mild dysplasia (Figure 6), these abnormalities were confined to the lower third of the epithelium; in moderate dysplasia, they were present in the lower two thirds of the epithelium; and in severe dysplasia (Figure 7), they involved the upper third of the epithelium as well. Full-thickness involvement of the epithelium (defined as carcinoma-in-situ by some) was included under severe dysplasia. Dysplasia not otherwise specified was diagnosed when dysplasia was present but could not be graded accurately because of poor tissue orientation or other artifact.

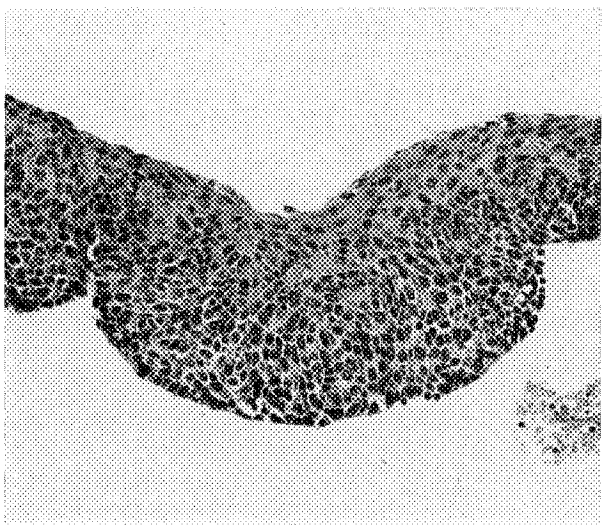

Figure 7. Severe squamous dysplasia, showing atypical nuclei and abnormal maturation throughout the epithelium (original magnification, ×200).

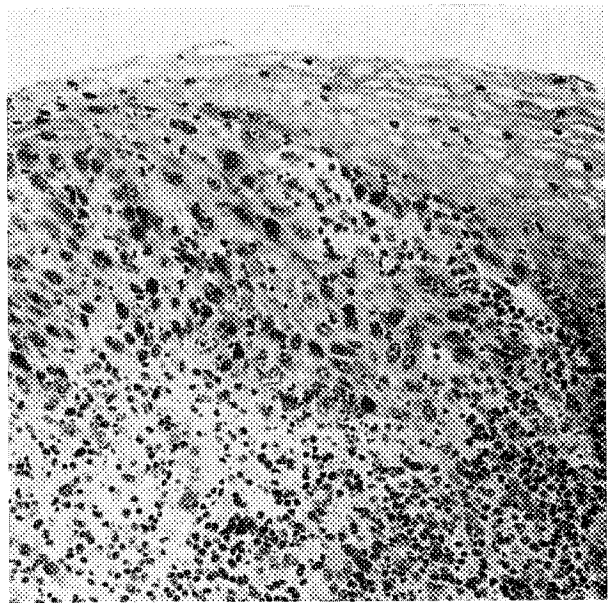

Figure 8. Intramucosal squamous cancer. A tongue of neoplastic squamous cells is invading the lamina propria. The adjacent epithelium (on the right) appears normal (original magnification, ×200).

Squamous cancer. Malignant squamous cells were present that had invaded through the basement membrane (Fig. 8). When invasion was confined to the lamina propria or muscularis mucosa, it was defined as intramucosal carcinoma.

Analysis

For each patient, a worst esophageal diagnosis was determined using the hierarchy of invasive cancer > dysplasia > esophagitis > acanthosis > normal.

Results

A total of 1567 satisfactory squamous biopsies were obtained, including 395 clinical biopsies, 562 formalin-fixed research biopsies, and 610 alcohol-fixed research biopsies. Seven hundred fifty-four (91%) of the 833 endoscoped patients had at least one satisfactory squamous esophageal biopsy.

Table 1 shows the results of the 1567 satisfactory squamous biopsies. The distributions of diagnoses of the formalin-fixed and alcohol-fixed research biopsies were similar, and, therefore, only the combined data are shown. Overall, 16% of the biopsies contained subepithelial lamina propria.

We classified 1114 (71.1%) of the 1567 biopsies as

Table 1. Histologic Diagnoses of 1567 Squamous Esophageal Biopsies from Linxian, China

Type of biopsy	No. of biopsies	Normal	Atrophy	Acanthosis	Esophagitis*				Dysplasia†					Cancer†
					Mild	*Moderate*	*Severe*	*Total*	*Mild*	*Moderate*	*Severe*	*Not otherwise specified*	*Total*	
Research	1172	915	0	80	19	4	1	24	69	31	20	7	127	26
		(78.1)	(0.0)	(6.8)	(1.6)	(0.3)	(0.1)	(2.0)	(5.9)	(2.6)	(1.7)	(0.6)	(10.8)	(2.2)
Clinical	395	199	0	23	13	5	6	24	41	20	42	17	120	29
		(50.4)	(0.0)	(5.8)	(3.3)	(1.3)	(1.5)	(6.1)	(10.4)	(5.1)	(10.6)	(4.3)	(30.4)	(7.3)
Total	1567	1114	0	103	32	9	7	48	110	51	62	24	247	55
		(71.1)	(0.0)	(6.6)	(2.0)	(0.6)	(0.4)	(3.1)	(7.0)	(3.3)	(4.0)	(1.5)	(15.8)	(3.5)

Data are number of biopsies; row percentages are shown in parentheses.

* Without dysplasia or cancer.

† With or without esophagitis.

normal. Five of these biopsies had elongated lamina propria papillae without accompanying basal cell hyperplasia, and 67 of them showed basal cell hyperplasia without elongated papillae.

We saw no examples of atrophy as defined above. Acanthosis was uncommon but not rare, diagnosed in 103 (6.6%) of the biopsies.

Esophagitis was the worst diagnosis in 48 (3.1%) of the biopsies. However, among biopsies showing dysplasia or cancer that were available for a second review, 20/116 (17%) with squamous dysplasia and 18/23 (78%) with squamous cancer showed accompanying esophagitis. Of the 48 biopsies with a worst diagnosis of esophagitis, 32 (67%) were graded as mild, 9 (19%) as moderate, and 7 (15%) as severe esophagitis. Among these biopsies, 8 (17%) showed elongated papillae and basal cell hyperplasia, 24 (50%) contained intraepithelial neutrophils, 3 (6%) contained intraepithelial eosinophils, and 19 (40%) showed lamina propria inflammation.

We diagnosed 247 (15.8%) of our biopsies as squamous dysplasia. Of these, 110 (45%) were graded as mild, 51 (21%) as moderate, 62 (25%) as severe, and 24 (10%) as dysplasia not otherwise specified. Of the 62 severely dysplastic biopsies, 18 showed full-thickness dysplasia and would have been called carcinoma-in-situ if that category had been used.

We had 55 biopsies of squamous cancer, 3.5% of our total biopsies. Thirty-one (56%) of these appeared to be intramucosal cancer, and 24 (44%) appeared to be more deeply invasive.

Greater proportions of the clinical biopsies showed cancer, dysplasia, or esophagitis, whereas more of the research biopsies showed normal mucosa.

Table 2 classifies the 754 patients by their worst esophageal diagnosis: 426 (56.5%) had normal mucosa, 87 (11.5%) showed acanthosis, 35 (4.6%) had esophagitis, 171 (22.7%) had squamous dysplasia, and 35 (4.6%) had invasive squamous cancer. This distribution of diagnoses did not differ significantly by treatment group,[9] and, therefore, only the combined data are shown.

Discussion

Since 1979, Crespi, Munoz et al.[10–13] and others using their methods[14,15] have described high prevalences of histologic esophagitis, acanthosis, atrophy, and dysplasia in Iranian and Chinese populations that have high esophageal squamous cancer rates and little or no evidence of esophageal reflux. They have proposed that some of these histologic findings may represent stages in the natural history of esophageal cancer in these populations.[11] An endoscopic survey conducted as a part of the Linxian nutrition intervention trials gave us an opportunity to make an independent study of esophageal histology in such a high-risk Chinese population.

Development of Histologic Criteria

Realizing that there might be distinctive histologic findings, we first reviewed several hundred biopsies to look for morphologic patterns before settling on criteria for histologic categories. We made the following observations:

Epithelial thickness. The thickness of the squamous epithelium in our biopsies formed a continuous spectrum, with no distinct steps between thin, normal, and thick epithelia. Thus, any categorization of cases based on epithelial thickness had to involve arbitrary divisions. We decided to record three thickness categories, and we tried to make our divisions as practical, objective, and reproducible as possible. We separated atrophy from normal epithelium by counting cell layers, as has been reported previously in Chinese studies,[6] because we found we could count the lower cell

Table 2. Worst Esophageal Diagnoses in 754 Endoscoped Patients from Linxian, China

No. of patients	Normal	Atrophy	Acanthosis	Esophagitis				Dysplasia					Cancer
				Mild	*Moderate*	*Severe*	*Total*	*Mild*	*Moderate*	*Severe*	*Not otherwise specified*	*Total*	
754	426	0	87	24	5	6	35	80	35	44	12	171	35
	(56.5)	(0.0)	(11.5)	(3.2)	(0.7)	(0.8)	(4.6)	(10.6)	(4.6)	(5.8)	(1.6)	(22.7)	(4.6)

Data are number of patients; row percentages are shown in parentheses.

layers of the squamous epithelium with reasonable accuracy. However, counting cell layers to separate acanthosis from normal epithelium was not accurate or reproducible, so we divided these categories by a simple microscopic measurement of the epithelial thickness.

We noticed at least two artifacts that could affect our estimates of epithelial thickness. Loss of surface epithelium during the biopsy procedure (Figure 9) was a common and potentially significant problem caused by the adherence of varying amounts of superficial epithelium to the biopsy forceps during tissue removal. Because it was impossible to know how much superficial tissue had been lost, it was not possible to compensate for this artifact when estimating epithelial thickness. Suboptimal orientation of biopsy tissue, which could cause an overestimation of epithelial thickness, was a less common problem, and compensation for this artifact usually could be made.

A few biopsies showed a thin epithelium overlying granulation tissue (Figure 10), an appearance suggesting epithelial regeneration over a mucosal erosion. We decided not to categorize the apparently changing epithelial thickness of these biopsies, all of which were diagnosed as esophagitis because of inflammatory cell infiltrates. We also saw a few dysplastic biopsies that were fewer than 10-cells thick (Figure 11); we diagnosed these biopsies as squamous dysplasia.

Epithelial clear cell change. Sixty-eight percent of our biopsies contained at least some enlarged spinous cells with abundant clear cytoplasm (Figure 12), an appearance we called clear cell change. The number and location of such cells varied widely, and we could not identify a pattern that was distinctive enough to be given a separate histologic diagnosis. In some biopsies, clear cells were seen preferentially at the lateral and/or superficial edges of the histologic sections, suggesting a relationship to the completeness of fixation or fluid exchange during tissue processing. A clear cell appearance was also seen regularly in poorly oriented squamous cells viewed *en face,* suggesting that it was sometimes an artifact of orientation.

Papillary height. Estimates of the height of lamina propria papillae relative to total epithelial thickness were necessarily affected by artifacts such as loss of surface epithelium or oblique cutting of biopsies. Our

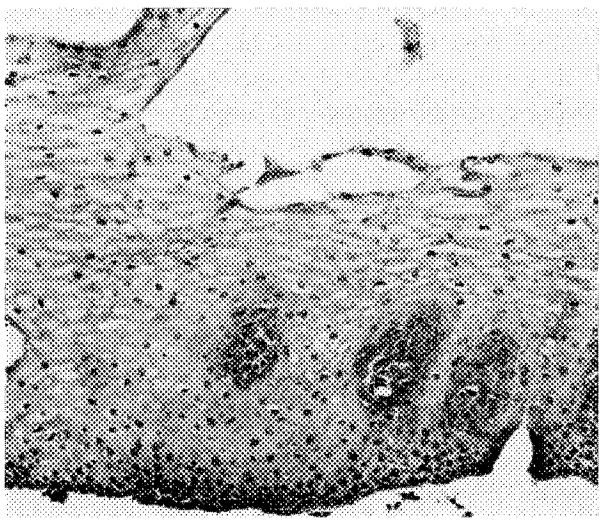

Figure 9. Artifactual loss of surface epithelium. This epithelium has been torn, probably during removal from the biopsy forceps. On the left it is acanthotic, but on the right, where the surface layers have been lost, it appears to have a normal thickness (original magnification, ×200).

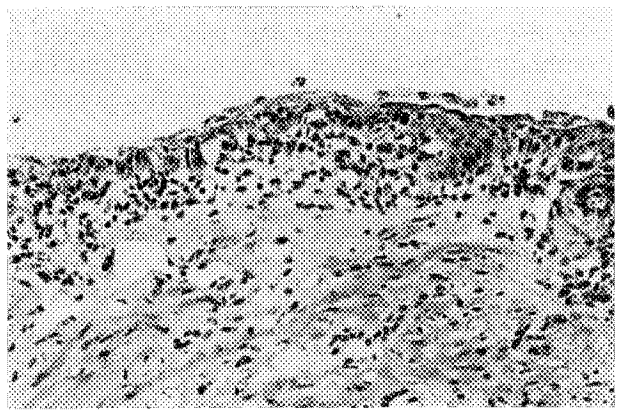

Figure 10. This biopsy shows a thin regenerating epithelium overlying granulation tissue and fibrosis (original magnification, ×200).

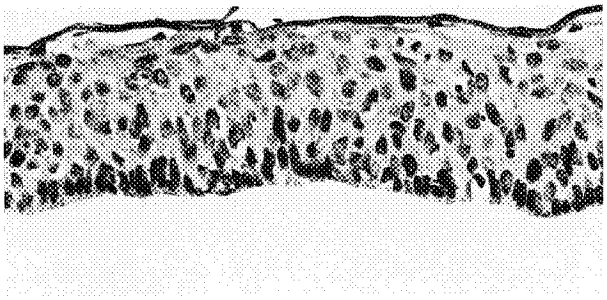

Figure 11. This dysplastic biopsy has fewer than 10 cell layers (original magnification, ×200).

estimates of papillary height formed a continuous spectrum. We decided to follow accepted criteria for reflux esophagitis[4,7] and require that papillae extend into the upper third of the epithelium and be accompanied by basal cell hyperplasia to be diagnostic of esophagitis. Elongated papillae and basal cell hyperplasia were not used to diagnose esophagitis in biopsies from the distal 2.5 cm of the esophagus, because they can be normal features in this area.[5]

Congestion of papillary capillaries. Capillary congestion and hemorrhage within lamina propria papillae has been described as a possible criterion for reflux esophagitis,[16] and it was mentioned as a criterion for esophagitis in previous endoscopic surveys in Linxian.[11–13] We found papillary vascular congestion in 32% of our biopsies. Most of these biopsies showed no other evidence of esophagitis, and many biopsies with clear esophagitis did not show this feature. It appeared that such congestion was usually an artifact of the biopsy procedure, so we did not use this as a criterion for diagnosing esophagitis.

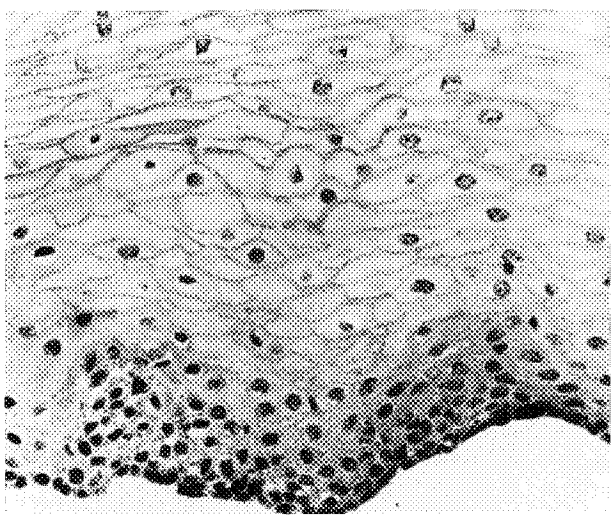

Figure 12. Clear cell change. The cells above the basal zone have abundant clear cytoplasm (original magnification, ×400).

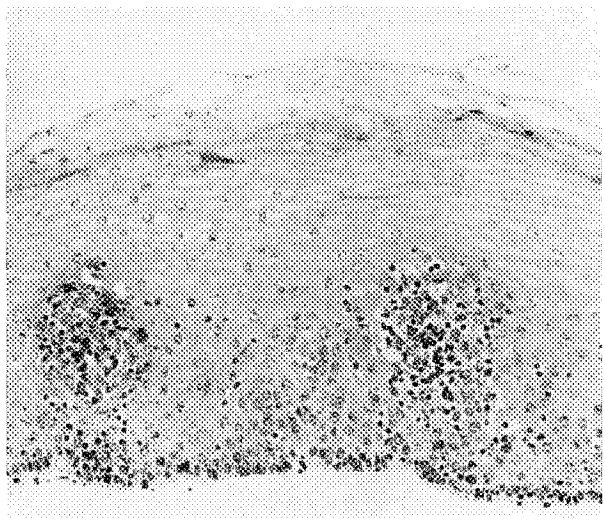

Figure 13. Focal aggregates of epithelial lymphocytes are seen around two lamina propria papillae. This was an occasional finding of uncertain significance (original magnification, ×200).

Basal cell hyperplasia We used Johnson et al.'s definition of the basal zone,[8] which we found to be simple and reproducible. Basal zone thickness could be estimated only in nondysplastic biopsies. As with measurements of papillary height, estimates of basal zone thickness relative to total epithelial thickness were influenced by artifacts that affected epithelial thickness measurements. Following accepted criteria for reflux esophagitis,[4,7] we decided to consider a basal zone greater than 15% (about one sixth) of the epithelial thickness to be abnormal, and we required that this basal cell hyperplasia be accompanied by elongation of the lamina propria papillae for a diagnosis of esophagitis to be made.

Epithelial inflammation. Several authors have documented that lymphocytes and Langerhans' cells are normal components of the esophageal squamous epithelium.[7,17,18] We found scattered intraepithelial lymphocytes in over 98% of our biopsies. We also found these lymphocytes in 100% of 63 biopsies from 19 asymptomatic Canadian patients who had been previously described[5] (slides provided by Dr. Wilfred M. Weinstein of UCLA). We decided that these cells were a normal finding, so we did not interpret them as histologic evidence of esophagitis. In addition to these scattered lymphocytes, a few biopsies showed focal aggregates of epithelial lymphocytes adjacent to lamina propria papillae (Figure 13). Usually, only one or two papillae in a biopsy were involved. We were uncertain of the significance of this finding, especially in biopsies that had been incubated for an hour before fixation, so we recorded its occurrence but did not include it as a criterion for esophagitis.

In addition to epithelial lymphocytes, 74% of our biopsies contained compressed nuclear material of uncertain origin that appeared squeezed between adjacent epithelial cells and was not associated with identifiable cytoplasm on the hematoxylin-and eosin-stained slides (Figure 14). This nuclear material, which we called "squiggle cells", was often elongated and/or fragmented, and occasionally appeared lobed. Some examples appeared morphologically consistent with Langerhans' cells.[17] We reviewed the 63 biopsies from asymptomatic Canadian patients and found similar material in 71% of them. We also stained slides from eight Linxian biopsies containing a representative spectrum of squiggle cells with chloracetate esterase (to stain neutrophils and eosinophils), leukocyte common antigen (to stain lymphocytes), Leu 26 (a pan B-cell marker), and CD 45 (a pan T-cell marker). We found that most of the compressed nuclear material stained as T-lymphocytes or did not stain at all, findings similar to those of another recent report.[19] Only rare squiggle cells stained as neutrophils, and these were always accompanied by clearly identifiable intraepithelial neutrophils in the same tissue sections. We concluded that although a few squiggle cells might be neutrophils or eosinophils, the great majority were most probably T-lymphocytes, Langerhans' cells, or degenerated nuclear material. We decided to diagnose intraepithelial neutrophils and eosinophils only when they were clearly identifiable, as such in Figure 4, and we did not accept compressed nuclear material as evidence of epithelial inflammation.

Lamina propria inflammation. Many authors consider mononuclear inflammatory cells in the lamina propria to be a nonspecific finding in the esophagus, and, thus, do not recognize their presence as a criterion for reflux esophagitis.[4,7] Other authors have considered a "slight lymphoplasmacytic and polymorphonuclear infiltrate and edema of the submucosa" to be diagnostic of esophagitis in high-risk areas such as Linxian.[10-13] After reviewing the initial Linxian biopsies and the biopsies from asymptomatic Canadian patients, we decided on an intermediate position, that scattered lymphocytes, plasma cells, and eosinophils and follicular clusters of lymphoid cells were probably normal findings in the lamina propria, but that dense nonfollicular infiltrates of mononuclear inflammatory cells (Figure 5) or easily recognizable infiltrates of neutrophils should be considered histologic evidence of esophagitis.

Squamous dysplasia and squamous cancer. The histologic criteria and grading of squamous dysplasia and squamous cancer are fairly well standardized, but opinions may differ on the use of the term carcinoma-in-situ. We feel that the most logical and reproducible point to separate squamous dysplasia and cancer is invasion of neoplastic epithelial cells through the basement membrane. Therefore, we did not use the term carcinoma-in-situ, and we required invasion to make a diagnosis of cancer.

Comparisons with Previous Studies

Our results (Tables 1 and 2) show a different distribution of esophageal squamous histologic diagnoses than has been previously reported from this population.

When comparing our findings with those of Crespi and Munoz[11-13] or Yang and Qiu,[14,15] it should be noted that our protocols differed from theirs in several ways: all of our patients had previous cytologic evidence of dysplasia; our protocols for biopsy site selection may have differed somewhat (but probably not greatly) from theirs; and many of our biopsies were incubated for 1 hour for tritiated thymidine incorporation before fixation.

Acknowledging these differences in protocols and methods, it still appears that the major factor causing our results to differ from those of other authors was differences in histologic criteria. Crespi et al.[10-13] do not state histologic criteria for any diagnostic categories except esophagitis, and their descriptions of esophagitis are brief and not sufficiently detailed for some comparisons. Qiu et al.[14,15] describe their diagnostic categories more completely. In all of these reports,[10-15] it is unclear how often esophagitis occurred alone, as the patient's worst diagnosis, and how often it accompanied dysplasia or cancer.

We found no biopsies in the current material that fit the common Chinese definition of atrophy. This is similar to what Qiu et al.[14,15] found in Huixian, a county near Linxian with a similar high incidence of esophageal cancer, but it is different from Crespi's or Qiu's findings in Linxian.[11,15] This difference probably reflects different criteria; for example, Qiu's description of atrophy sounds like a thin epithelium overlying granulation tissue (Figure 10), an appearance we diagnosed as esophagitis. In addition, previous authors may have included thin dysplastic epithelia (Figure 11) twice in their prevalence figures, both as atrophy and as dysplasia, whereas we counted these cases only once, as dysplasia.

Our definition of acanthosis was purely an arbitrary one, designed to separate the thickest epithelia from the rest, without regard for the presence of clear cell change, which appeared to be a common and nonspecific finding in biopsies of all thicknesses. By our definition, acanthosis was uncommon but not rare. Crespi's finding that 77% of patients in this population showed clear cell acanthosis[11-13] must have relied on a significantly different division point to separate thick from normal biopsies and/or must have based this

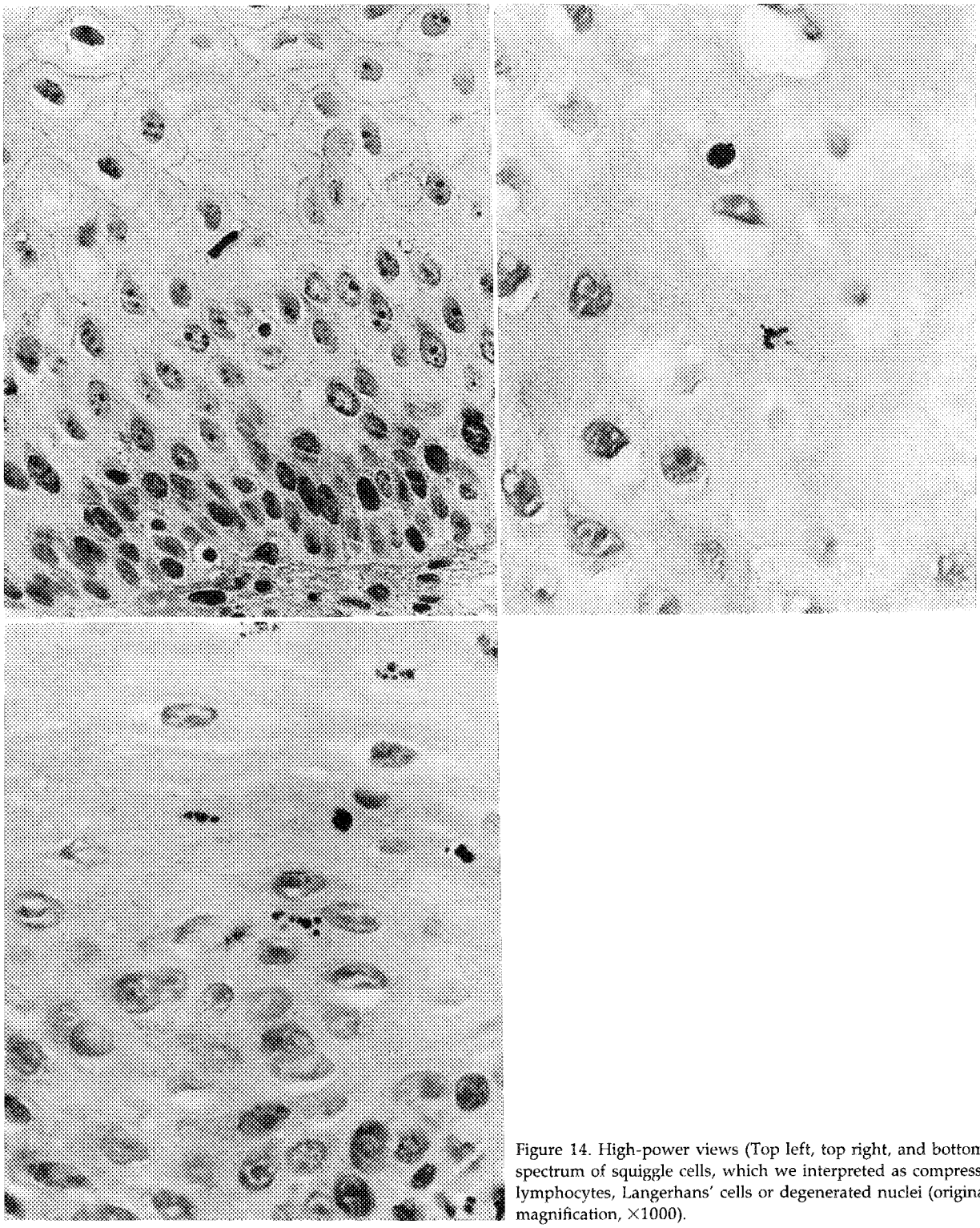

Figure 14. High-power views (Top left, top right, and bottom left) of a spectrum of squiggle cells, which we interpreted as compressed lymphocytes, Langerhans' cells or degenerated nuclei (original magnification, ×1000).

diagnosis on the finding of clear cell change, irrespective of the epithelial thickness.

Several aspects of the diagnosis of esophagitis deserve comment: We saw many biopsies with papillae in the middle third of the epithelium, but only a few in which they reached the upper third. Crespi et al.[10–13] do not state papillary height criteria and give no data on how many of their biopsies showed abnormally elongated papillae. The finding by Qiu et al.[14,15] that 81% of their biopsies from Huixian showed abnormal papillary

upgrowth probably reflects their decisions that papillae in the middle third of the epithelium are abnormal and that Kobayashi et al.'s criteria[20] identify abnormally elongated papillae in obliquely cut sections.

Crespi et al.[10–13] do not mention basal cell hyperplasia as a criterion for esophagitis. Qiu et al.,[14,15] requiring that the basal zone be greater than 50% of epithelial thickness to diagnose basal cell hyperplasia, identified this finding in 31% of patients from Linxian and 74% of patients from Huixian, prevalences that are much greater than what we found in our biopsies.

We found basal cell hyperplasia associated with elongated papillae in only eight biopsies. Since there may be regional differences in papillary height, even within individual biopsies, it may be that larger biopsies, with more papillae, would have allowed more specimens to have been diagnosed as esophagitis.

The decisions about histologic criteria for esophagitis that affected our results the most were the decisions about epithelial and lamina propria inflammation discussed above. If we had accepted scattered intraepithelial lymphocytes as evidence of esophagitis, virtually all of our biopsies would have received this diagnosis. If all squiggle cells had been accepted as epithelial inflammation, over 70% of our biopsies would have been diagnosed as esophagitis, based on this criterion alone. Accepting scattered mononuclear cells as lamina propria inflammation would also have dramatically increased our prevalence of esophagitis. By not including these findings as evidence of inflammation, only 4.6% of our patients had a worst diagnosis of esophagitis. Crespi et al.,[10–13] who appear to have relied heavily on lamina propria infiltrates of mononuclear cells for diagnosing and grading esophagitis, found histologic esophagitis in 64% of their patients in Linxian. Qiu et al.,[15] who based their grading of esophagitis on epithelial lymphocytes and neutrophils, reported epithelial infiltration by neutrophilic granulocytes in 75% of their patients in Huixian. We believe that in this latter series, they must have included our squiggle cells as neutrophils.

We found basal cell hyperplasia together with elongated lamina propria papillae (8 biopsies), with epithelial inflammation (4 biopsies), and alone, with neither of these other findings (67 biopsies). We assume that basal cell hyperplasia was a response to epithelial injury in the first two settings, but we wonder about its meaning when found alone: was it a response to a noninflammatory epithelial injury, or was it a primary proliferation of basal cells, perhaps an early stage of neoplasia? It will be interesting to follow these patients, to see if basal cell hyperplasia in any of these settings predicts an increased risk of developing dysplasia or cancer.

In our series, 23% of the patients had a worst squamous diagnosis of dysplasia, and an additional 4.6% had invasive squamous cancer. These prevalence figures are greater than those reported from Linxian by Crespi et al. (8% dysplasia and 0.9% squamous cancer)[11–13] or by Qiu et al. (17% dysplasia and 1.8% squamous cancer),[15] probably because our survey was limited to patients with a previous cytologic diagnosis of dysplasia.

The fact that only 27% of our patients showed histologic dysplasia or cancer may be due to a combination of factors, including regression of dysplastic lesions in the 4 years between the cytologic and endoscopic examinations, insufficient sampling by the endoscopic biopsies, and false-positive initial cytologic diagnoses. Insufficient biopsy sampling was probably not the major factor in this discrepancy, because another recent study from Linxian has shown that most histologic dysplasia and cancer of the squamous esophagus in this population is associated with endoscopically visible focal lesions similar those targeted in the 1987 biopsy protocol.[21] Regarding the cytologic diagnoses, the cytologic categories and criteria used in the 1983 screening were developed and have been used almost exclusively in China, and they have not yet been correlated carefully with the cytologic categories used in other countries or with same-site biopsy diagnoses.[3,22] Such correlation studies and longitudinal follow-up studies are needed to help clarify discrepancies between Chinese balloon cytology diagnoses and endoscopic biopsy findings.

In summary, the 1987 endoscopic survey in Linxian allowed us to examine a wide range of squamous esophageal biopsies from a Chinese population that has a high risk of developing squamous esophageal cancer. Our results show a different distribution of diagnoses than has been previously reported from this population. We think that the major reason for this discrepancy was differences in histologic criteria. In our survey, seemingly small differences in criteria could cause large differences in apparent disease prevalence. By our criteria, histologic esophagitis and atrophy are uncommon findings in Linxian, raising questions about their significance as precursor lesions of esophageal cancer in this population. Achieving more standardized definitions of diagnostic categories and documenting the relationship of these categories to the development of cancer in follow-up studies should improve our understanding of esophageal carcinogenesis.

References

1. Li JY. Epidemiology of esophageal cancer in China. *Natl Cancer Inst Monogr* 1982; 62:113–20.

2. Li B, Taylor PR, Li JY, Dawsey SM, Wang W, Tangrea JA, et al. Linxian nutrition intervention trials: design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1993; 3:577–85.
3. Shen Q, Liu SF, Dawsey SM, Cao J, Zhou B, Wang DY, et al. Cytologic screening for esophageal cancer: results from 12,877 subjects from a high-risk population in China. *Int J Cancer* 1993; 54:185–8.
4. Ismail-Beigi F, Horton PF, Pope CE. Histological consequences of gastroesophageal reflux in man. *Gastroenterology* 1970; 58:163–74.
5. Weinstein WM, Bogoch ER, Bowes KL. The normal human esophageal mucosa: a histological reappraisal. *Gastroenterology* 1975; 68:40–4.
6. Tumor Prevention, Treatment and Research Group, Chengchow, Honan; Esophageal Cancer Research Group, Chinese Academy of Medical Sciences, Peking; and Linhsien County People's Hospital, Honan. Pathology of early esophageal squamous carcinoma. *Chinese Med J* 1977; 3:180–92.
7. Mitros FA. Inflammatory and neoplastic diseases of the esophagus. In: Appleman HD, (editor). Pathology of the esophagus, stomach and duodenum. New York: Churchill Livingstone, 1984:1–35.
8. Johnson LF, Demeester TR, Haggitt RC. Esophageal epithelial response to gastroesophageal reflux: a quantitative study. *Dig Dis* 1978; 23:498–509.
9. Dawsey SM, Wang GQ, Taylor PR, Li JY, Blot WJ, Li B, et al. Effects of nutritional intervention on the prevalence of histologic dysplasia and early cancer of the esophagus and stomach: results from the dysplasia trial in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 1994; 3:167–72.
10. Crespi M, Munoz N, Grassi A, Aramesh B, Amiri G, Mojtabai A, et al. Oesophageal lesions in northern Iran: a premalignant condition? *Lancet* 1979; 2:217–21.
11. Munoz N, Crespi M, Grassi A, Wang GQ, Shen Q, Li ZC. Precursor lesions of oesophageal cancer in high-risk populations of Iran and China. *Lancet* 1982; 1:876–9.
12. Crespi M, Munoz N, Grassi A, Shen Q, Wang GQ, Lin JJ. Precursor lesions of oesophageal cancer in a low-risk population in China: comparison with high-risk populations. *Int J Cancer* 1984; 599–602.
13. Crespi M, Grassi A, Munoz N, Wang GQ, Yang GR. Endoscopic features of suspected precancerous lesions in high-risk areas for esophageal cancer. *Endoscopy* 1984; 16:85–91.
14. Yang GR, Qiu SL (published as Guanrei Y, Songliang Q, but the authors' family names are Yang and Qiu). Endoscopic surveys in high-risk and low-risk populations for esophageal cancer in China with special reference to precursors of esophageal cancer. *Endoscopy* 1987; 19:91–5.
15. Qiu SL, Yang GR. Precursor lesions of esophageal cancer in high-risk populations in Henan Province, China. *Cancer* 1988; 62:551–7.
16. Geboes K, Desmet V, Vantrappen G, Mebis J. Vascular changes in the esophageal mucosa: an early histologic sign of esophagitis. *Gastrointest Endosc* 1980; 26:29–32.
17. Geboes K, De Wolf-Peeters C, Rutgeerts P, Janssens J, Vantrappen G, Desmet V. Lymphocytes and Langerhans cells in the human oesophageal epithelium. *Virchows Arch [Pathol Anat]* 1983; 401:45–55.
18. Al Yassin TM, Toner PG. Langerhans cells in the human oesophagus. *J Anat* 1976; 122:435–45.
19. Mangano MM, Antonioli DA, Schnitt SJ, Wang HH. Nature and significance of cells with irregular nuclear contours in esophageal mucosal biopsies. *Mod Pathol* 1992; 5:191–6.
20. Kobayashi S, Kasugai T. Endoscopic and biopsy criteria for diagnosis of esophagitis with fiberoptic endoscopy. *Dig Dis* 1974; 4:345–52.
21. Dawsey SM, Wang GQ, Weinstein WM, Lewin KJ, Liu FS, Wiggett S, et al. Squamous dysplasia and early esophageal cancer in the Linxian region of China: distinctive endoscopic lesions. *Gastroenterology* 1993; 105:1333–40.
22. Dawsey SM, Yu Y, Taylor PR, Li JY, Shen Q, Shu YJ, et al. Esophageal cytology and subsequent risk of esophageal cancer: a prospective follow-up from Linxian, China. *Acta Cytol* 1994; 38:183–192.